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DN 105:168806  
TI Directed selection of differentiation mutants of *Streptomyces noursei*  
using chemostat cultivation  
AU Noack, D.  
CS Forschungsbereich Biowiss. Med., Dtsch. Akad. Wiss., Jena, DDR-6900, Ger.  
Dem. Rep.  
SO J. Basic Microbiol. (1986), 26(4), 231-9

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FUNDAMENTAL STUDIES OF URINARY TRACT INFECTION WITH *SERRATIA* INTERACTION  
BETWEEN BACTERIA OF DIFFERENT GENERA AND SPREADING OF R PLASMID TO  
*SERRATIA*.

AU MASU C  
CS DEP. UROL., HIROSHIMA UNIV. SCH. MED.  
SO MED J HIROSHIMA UNIV. (1986) 34 (4), 453-472.  
CODEN: HDIZAB. ISSN: 0018-2087.  
FS BA; OLD  
LA Japanese

Adaptive reversion of a frameshift mutation in *Escherichia coli*

AU Cairns, John; Foster, Patricia L.  
CS Dep. Cancer Biol., Harvard Sch. Public Health, Boston, MA, 02115, USA  
SO Genetics (1991), 128(4), 695-701  
CODEN: GENTAE; ISSN: 0016-6731

TI Modification in penicillin-binding proteins during in vivo development of  
genetic competence of *Haemophilus influenzae* is associated with a rapid  
change in the physiological state of cells

AU Dargis, M.; Gourde, P.; Beauchamp, D.; Foiry, B.; Jacques, M.; Malouin, F.  
CS Cent. Rech., Cent. Hosp., Ste-Foy, PQ, G1V 4G2, Can.  
SO Infect. Immun. (1992), 60(10), 4024-31

I Decreased susceptibilities to teicoplanin and vancomycin among  
coagulase-negative methicillin-resistant clinical isolates of  
staphylococci

AU Sieradzki, Krzysztof; Villari, Paolo; Tomasz, Alexander  
CS The Rockefeller University, New York, NY, 10021, USA  
SO Antimicrobial Agents and Chemotherapy (1998), 42(1), 100-107

Triclosan and antibiotic resistance in *Staphylococcus aureus*

AU Suller, M. T. E.; Russell, A. D.  
CS Pharmaceutical Microbiology, Welsh School of Pharmacy, Cardiff University,  
Cardiff, CF10 3XF, UK  
SO Journal of Antimicrobial Chemotherapy (2000), 46(1), 11-18  
CODEN: JACHDX; ISSN: 0305-7453

TI Augmentation of antibiotic resistance in *Salmonella typhimurium* DT104  
following exposure to penicillin derivatives

AU Carlson, S. A.; Ferris, K. E.  
CS National Animal Disease Center, Pre-harvest Food Safety and Enteric  
Disease Research Unit, Agricultural Research Service, USDA, Ames, IA, USA  
SO Veterinary Microbiology (2000), 73(1), 25-35

TI Augmentation of antibiotic resistance in *Salmonella typhimurium* DT104  
following exposure to penicillin derivatives

Original articles

## Triclosan and antibiotic resistance in *Staphylococcus aureus*

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Triclosan (2,4,4'-trichloro-2'-hydroxydiphenyl ether) is an antimicrobial agent used in hygiene products, plastics and kitchenware, and for treating methicillin-resistant *Staphylococcus aureus* (MRSA) outbreaks. *S. aureus* strains with low-level resistance to triclosan have emerged. It has been claimed that strains with decreased susceptibility to biocides may also be less susceptible to antibiotics. We tested the susceptibility of *S. aureus* clinical isolates to triclosan and several antibiotics. Triclosan MICs ranged between 0.025 and 1 mg/L. Some, but not all, strains were resistant to several antibiotics and showed low-level triclosan resistance. *S. aureus* mutants with enhanced resistance to triclosan ( $\leq 1$  mg/L) were isolated. In several cases this resistance was stably inherited in the absence of triclosan. These mutants were not more resistant than the parent strain to several antibiotics. Changes in triclosan MICs associated with the acquisition of a plasmid encoding mupirocin resistance were not observed, suggesting that the triclosan/mupirocin co-resistance seen in a previous study was not the result of a single resistance gene or separate genes on the same plasmid. The continuous exposure of a triclosan-sensitive *S. aureus* strain to sub-MIC concentrations of triclosan for 1 month did not result in decreased susceptibility to triclosan or to several antibiotics tested. Triclosan-induced potassium leakage and bactericidal effects on a triclosan-sensitive strain, a resistant strain and a strain selected for increased resistance were compared with those of non-growing organisms, exponentially growing organisms and organisms in the stationary phase. No significant differences between the strains were observed under these conditions despite their different MICs. Biocides have multiple target sites and so MICs often do not correlate with bactericidal activities. The ability of *S. aureus* to develop resistance to triclosan and the current view that triclosan may have a specific target in *Escherichia coli*, namely enoyl reductase, underline the need for more research on the mechanisms of action and resistance.

### Introduction

Triclosan (2,4,4'-trichloro-2'-hydroxydiphenyl ether) is a synthetic bisphenol antimicrobial agent, which is active against a wide range of Gram-positive and Gram-negative bacteria. It is included in many hygiene products, such as soaps and mouthwashes,<sup>1</sup> and is increasingly being incorporated into a range of items including toys, tea towels and chopping boards.<sup>2</sup> In 1998, it was recommended for the control of methicillin-resistant *Staphylococcus aureus* (MRSA)<sup>3</sup> after being successfully used to control MRSA outbreaks in a neonatal nursery<sup>4</sup> and cardiothoracic surgical unit<sup>5</sup> and to provide an alternative to expensive vancomycin administration.<sup>6</sup>

*S. aureus* strains have emerged that exhibit low-level resistance to triclosan. Cookson *et al.*<sup>7</sup> isolated MRSA strains with MICs of 2–4 mg/L from patients treated with daily triclosan baths; this compares with MICs of 0.01–0.1 mg/L for sensitive strains. In a recent study, 7.5% of *S. aureus* clinical isolates were found to have triclosan MICs of at least 1 mg/L, although differences between MRSA and methicillin-susceptible *S. aureus* (MSSA) strains were not evident.<sup>8</sup> Furthermore, Cookson *et al.*<sup>7</sup> found triclosan resistance to be transferable in association with plasmid-mediated mupirocin resistance.

Triclosan has been regarded as a biocide rather than an antibiotic and, as such, has been thought to have numerous intracellular and cytoplasmic target sites.<sup>9</sup> This view has

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recently been challenged with evidence that triclosan may act on a specific target, the enoyl reductase enzyme, which is involved in the synthesis of fatty acids.<sup>10</sup> However, McDonnell & Pretzer<sup>11</sup> maintain that, although enoyl reductase may be the major target site, triclosan also affects other components. If triclosan does act as a specific inhibitor, then it is likely that strains with higher levels of resistance will emerge, as is the case with antibiotics. To date, resistance levels amongst *S. aureus* appear to be low; the clinical importance of this remains to be seen.

In this study, a range of *S. aureus* clinical isolates were tested for their susceptibilities to triclosan and several antibiotics. Triclosan-induced bactericidal effects and leakage of intracellular material were compared in a resistant strain, a sensitive strain and a mutant that had been selected for increased resistance to triclosan. The susceptibility of triclosan-resistant mutants to various antibiotics, compared with the sensitive parent strain, was determined, and the stability of the resistance phenotype tested. The effect of continuous exposure of a sensitive strain to a low concentration of triclosan was also examined in terms of changes to triclosan and/or antibiotic sensitivity. Finally, we transferred plasmids encoding mupirocin resistance to a susceptible recipient in order to test whether triclosan resistance was encoded on these plasmids, as previously claimed.<sup>7</sup>

## Materials and methods

### Bacterial strains and antibiotic susceptibility testing

*S. aureus* clinical isolates 11777, 9543 and 16565 were supplied by Dr E. G. M. Power of St Thomas' Hospital, London, UK. All other isolates were obtained from Mr Alan Paull of the University Hospital of Wales, Cardiff, UK. All isolates had been tested before receipt and those described as MRSA shown to be methicillin resistant. Antibiotic susceptibility was tested by Stokes' disc diffusion method<sup>12</sup> on nutrient agar (Oxoid, Basingstoke, UK) with *S. aureus* NCTC 6571 as control. Discs used were vancomycin 30 µg, gentamicin 10 µg, erythromycin 15 µg, penicillin 10 units, rifampicin 5 µg, novobiocin 5 µg, teicoplanin 30 µg, fusidic acid 5 µg, tetracycline 30 µg, methicillin 5 µg, chloramphenicol 30 µg, mupirocin 5 µg and streptomycin 10 µg. Nutrient agar was used as the culture medium, as it had been used in other studies with these organisms. However, no differences were observed in resistance patterns when DST agar (Oxoid) was used as the test medium.

### Determination of disinfectant MICs

MICs of triclosan (Ciba Geigy, Manchester, UK), chlorhexidine diacetate (Sigma, Poole, UK), cetylpyridinium chloride (BDH Chemicals, Poole, UK), triclocarban (Aldridge, Milwaukee, WI, USA) and dibromopropamide isethionate (Rhone-Poulenc Rohrer, Dagenham, UK)

were determined on nutrient agar plates containing a range of disinfectant concentrations. One microlitre of an overnight culture of the test organism (diluted 10-fold; equivalent to c.  $5 \times 10^4$  cfu/spot) was used to inoculate the surface of a nutrient agar plate, using a multipoint inoculator (Denley, Billingham, UK). Plates were incubated for 24 h at 37°C and the MIC taken as the lowest concentration that inhibited growth. Triclosan susceptibility was tested over the concentration range 0–5 mg/L.

### Transfer of mupirocin resistance

*S. aureus* clinical isolates 11777, 9543, 16565 and HLMR exhibit plasmid-encoded high-level resistance to mupirocin (MIC > 250 mg/L) and were used as plasmid donors in transfer experiments. *S. aureus* RF2, which is sensitive to mupirocin but resistant to tetracycline, was used as the recipient organism. Donor and recipient bacteria were grown separately overnight in nutrient broth (Oxoid) at 37°C and then mixed in equal volumes. Two millilitres of this mixture was then filtered through a 0.2 µm cellulose nitrate filter. The filter was placed on the surface of DST agar, incubated for 18 h at 37°C and then resuspended in 2 mL of one-tenth strength nutrient broth. DST agar containing mupirocin 5 mg/L (a gift from SmithKline Beecham, Betchworth, UK) and tetracycline 5 mg/L (Sigma) was used to isolate transconjugant bacteria (RF2 with mupirocin-resistance plasmid). The MICs of triclosan, chlorhexidine, cetylpyridinium chloride, triclocarban and dibromopropamide isethionate for the transconjugants were then determined.

### Isolation of triclosan-resistant mutants and stability testing

Absorbent 6 mm discs pre-soaked in triclosan 10 mg/L were placed on the surface of a nutrient agar plate seeded with an overnight culture of *S. aureus* NCTC 6571. After incubation for 48 h at 37°C, colonies that appeared within the zone of inhibition surrounding the disc were tested for decreased triclosan susceptibility. The stability of this resistance was then examined by serial overnight subculturing in nutrient broth for 14 days, with MIC testing every 48 h.

### Determination of antibiotic MICs

The MICs of vancomycin, penicillin, methicillin, erythromycin, gentamicin and tetracycline were determined using Etest strips (Cambridge Diagnostic Services, Cambridge, UK). An overnight culture of the test organism was used to seed the surface of a DST agar plate and, after incubation for 24 h at 37°C, results were interpreted according to the manufacturer's guidelines. The parent strain (NCTC 6571) and four triclosan-resistant mutants were tested to examine whether the triclosan resistance was associated with a decrease in antibiotic susceptibility.

### *Continuous exposure to triclosan at sub-MIC concentrations*

To test the premise that continuous exposure of bacteria to low concentrations of triclosan may result in organisms with decreased susceptibility to triclosan and antibiotics; *S. aureus* NCTC 6571 was grown overnight in nutrient broth containing triclosan 0.01 mg/L. Further subculturing was repeated on a daily basis, and the MICs of triclosan and a range of antibiotics were determined at intervals.

### *Determination of potassium leakage*

Cultures were grown on the surface of a nutrient agar plate at 37°C for 24 h. Cells were then emulsified in 5 mL of 0.9% NaCl (Sigma), washed three times by centrifugation and resuspended in 100 mL of 0.9% NaCl, with the appropriate concentration of triclosan, to give a bacterial concentration of  $10^9$  cfu/mL. At timed intervals, 10 mL was removed and filtered through a 0.2 µm cellulose nitrate filter to remove cellular material. The potassium concentration in the supernatant was then measured using an atomic absorption spectrophotometer. As control experiments, cells were incubated in triclosan-free NaCl solution (negative control) and treated with lysostaphin 100 mg/L (Sigma) for 30 min at 30°C and heated at 100°C for 10 min (positive controls).

### *Bactericidal effects of triclosan*

The effects of triclosan on stationary phase organisms, those in the exponential phase of growth and those from a non-growing culture were compared as follows. For the non-growing culture, reaction vessels were set up as with the potassium leakage protocol but with the final concentration of organisms adjusted to  $10^7$  cfu/mL. At timed intervals, 1 mL samples were taken and added to 9 mL of a neutralizing solution (2% lecithin and 5% Tween 80, both from Sigma). Surface counts of cfu were performed after serial dilution in phosphate-buffered saline (PBS), pH 7.6 (Sigma). Plates were incubated at 37°C for 24 h. For the stationary phase culture, an overnight culture was added directly to 100 mL of triclosan-containing 0.9% NaCl solution to give a concentration of  $10^7$  cfu/mL. For the exponential phase culture, 1 mL of the overnight culture was transferred into 9 mL nutrient broth and incubated for 4 h; 1 mL was then added to the reaction vessel to give a concentration of  $10^7$  cfu/mL.

## Results

### *Triclosan MICs and antibiotic resistance profiling*

The MICs of triclosan and the antibiotic resistance profiles for a range of *S. aureus* clinical isolates are shown in Table I. Triclosan MICs ranged between 0.025 and 1 mg/L (a

40-fold difference between the most sensitive and most resistant strains). Several strains, such as 11777, 9543 and 16565, showed resistance to a wide range of antibiotics and also exhibited low-level resistance to triclosan. However, other strains, such as MRSA 2 and 8, which were resistant to several antibiotics, were more susceptible to triclosan (MIC < 0.1 mg/L). *S. aureus* 50440 was resistant only to penicillin, but had an elevated MIC of triclosan (1 mg/L).

### *Transfer of mupirocin resistance*

Plasmids containing the determinant for mupirocin resistance were transferred from the donor strains 11777, 9543, 16565 and HLMR to the recipient strain RF2 at frequencies of between  $10^{-4}$  and  $10^{-5}$ . Decreases in susceptibility to triclosan or the other disinfectants, associated with the acquisition of this plasmid, were not observed in any of the transconjugants tested (20 from each donor-recipient mating).

### *Stability of triclosan resistance in mutants*

*S. aureus* mutants with enhanced triclosan resistance (MICs ≤ 1 mg/L) were isolated using the disc diffusion technique. The MICs of triclosan for 10 of these mutants (Table II) were determined after propagating the colonies in medium with or without triclosan. The triclosan MIC for mutants TM1-5 (1 mg/L) was 40 times greater than that for the parent strain (0.025 mg/L). The MIC of 1 mg/L for mutants TM1, 2 and 3 was stable whether the cells were propagated in triclosan-containing or triclosan-free medium. The MICs for mutants TM6-9 were 0.075, 0.4 and 0.4 and 1 mg/L, respectively. This resistance was lost when cells were grown in the absence of triclosan, indicating that it was unstable in the absence of a selective pressure. Mutant TM10 had a stable MIC of 0.75 mg/L. The stable triclosan-resistant mutants TM1, 2, 3 and 10 were subcultured daily in triclosan-free medium for 14 days. After this period, no reduction in MIC of triclosan was observed, indicating the stability of this resistance.

### *MICs of antibiotics for triclosan-resistant mutants*

The MICs of vancomycin, methicillin, penicillin, erythromycin, gentamicin and tetracycline for the parent strain *S. aureus* NCTC 6571 and mutant strains TM1, 2, 3 and 10 are shown in Table III. Enhanced resistance to triclosan in the mutant strains was not associated with an increase in MIC of any of the antibiotics tested, relative to their parent strain.

### *Continuous exposure to triclosan (sub-MIC)*

The exposure of *S. aureus* NCTC 6571 or MRSA 7 to triclosan at a sub-inhibitory concentration (0.01 mg/L) for 1 month did not result in decreased susceptibility to tri-

**Table I.** MICs of triclosan and antibiotic resistance profiles for *Staphylococcus aureus* strains

Staphylococcal strain	Antibiotic resistance profile	Triclosan MIC (mg/L)
NCTC 6571	—	0.025
Sau3	Fus, Gen, Rif	0.025
Sau2	Fus, Str	0.025
RF2	Pen, Tet, Str, Ery	0.025
MRSA 2	Pen, Met, Tet, Str, Ery	0.075
MRSA 3	Pen, Met, Tet, Str, Ery	0.1
MRSA 4	Pen, Met, Gen, Tet, Str, Ery	0.1
MRSA 5	Pen, Met, Tet, Str, Ery	0.4
MRSA 6	Pen, Met, Ery	0.1
MRSA 7	Pen, Met, Gen, Tet, Str, Ery	0.2
MRSA 8	Pen, Met, Tet, Str, Ery	0.075
MRSA 9	Pen, Met, Tet, Str, Ery	0.1
MRSA 11	Pen, Met, Gen, Ery	0.075
MRSA 12	Pen, Met, Ery	0.025
MRSA 13	Pen, Met, Ery	0.075
MRSA 15	Pen, Met	0.075
MRSA 11777	Pen, Met, Gen, Rif, Str, Ery, Mup	0.5
MRSA 9543	Pen, Met, Gen, Rif, Str, Ery, Mup	1
MRSA 16565	Pen, Met, Gen, Rif, Str, Ery, Mup	1
HLMR	Pen, Mup	0.075
Z60797	—	0.025
Z60815	Ery	0.075
Z60830	—	0.025
Z60836	Pen	0.1
Z60710	Pen	0.1
50434	Pen	0.025
50440	Pen	1
50256	Pen, Tet	0.1
50328	Pen	0.025
50325	Pen	0.3
50329	Pen	0.1
50332	—	0.1
50314	Pen	0.025

Ery, erythromycin; Fus, fusidic acid; Gen, gentamicin; Met, methicillin; Mup, mupirocin; Pen, penicillin G; Rif, rifampicin; Str, streptomycin; Tet, tetracycline.

closan, vancomycin, methicillin, penicillin, erythromycin, gentamicin or tetracycline.

#### *Triclosan-induced potassium leakage and bactericidal effects*

Intracellular potassium leakage resulting from exposure of *S. aureus* NCTC 6571, MRSA 9543 and TM1 to various concentrations of triclosan is shown in the Figure. Strain-to-strain variations in the rate of potassium leakage were not evident at triclosan concentrations of 2, 7.5 or 15 mg/L, despite the different MICs of the strains tested (0.025, 1 and 1 mg/L, respectively). Triclosan-induced rate of kill, as

judged by the recovery of surviving bacteria on solid agar after exposure to triclosan 7.5 mg/L (Table IV), also failed to reveal any significant differences between the three strains. This lack of correlation between MICs and bactericidal efficacy was observed in all the strains tested. Table IV also shows the anti-staphylococcal activities of triclosan on exponentially growing cultures, compared with stationary phase and non-growing cultures. It is clearly evident that the exponentially growing organisms are not more vulnerable to triclosan-induced loss of viability than non-growing organisms or those in the stationary phase. Similar results were obtained after exposure to triclosan 2 or 15 mg/L (data not shown).

## Staphylococcal triclosan resistance

**Table II.** Triclosan MICs of *Staphylococcus aureus* mutants selected for increased resistance to triclosan from strain NCTC 6571 (triclosan MIC 0.025 mg/L), determined after subculturing overnight in the presence or absence of triclosan and after repeated subcultures in its absence

Triclosan-resistant mutant	Overnight subculture		Repeated subcultures in absence of triclosan
	with triclosan	without triclosan	
TM1	1	1	1
TM2	1	1	1
TM3	1	1	1
TM4	1	1	NT
TM5	1	1	NT
TM6	0.075	0.025	NT
TM7	0.4	0.025	NT
TM8	0.4	0.025	NT
TM9	1	0.025	NT
TM10	0.75	0.75	0.75

NT, not tested.

**Table III.** MICs (mg/L) of various antibiotics (determined using Etest strips) for *S. aureus* NCTC 6571 and mutants selected for increased triclosan resistance

<i>S. aureus</i> strain	Triclosan	Vancomycin	Methicillin	Penicillin	Gentamicin	Erythromycin	Tetracycline
NCTC 6571	0.025	1	1	0.064	0.38	0.38	0.25
TM1	1	1.5	1	0.064	0.38	0.38	0.25
TM2	1	1.5	1	0.094	0.38	0.38	0.19
TM3	1	1.5	1	0.094	0.38	0.38	0.25
TM10	0.75	1.5	1	0.064	0.19	0.38	0.19

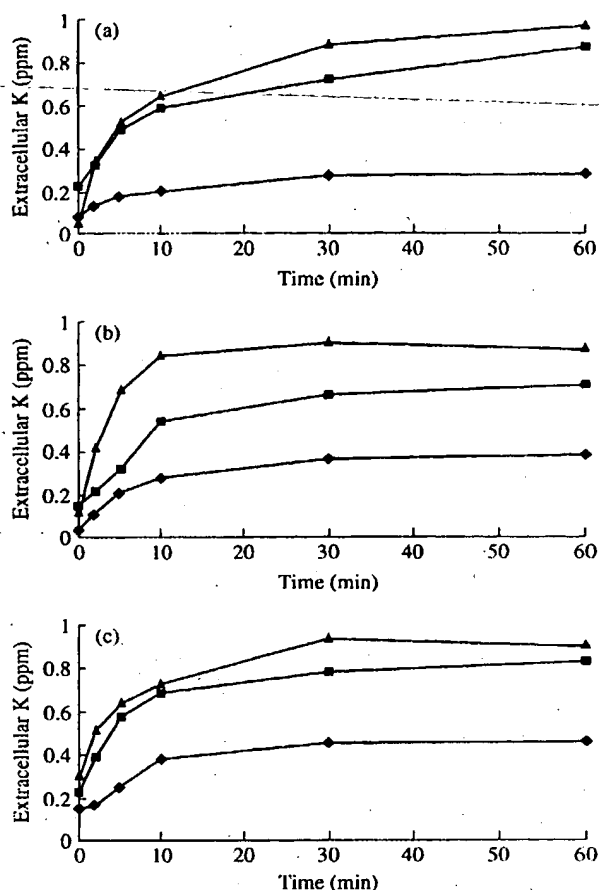
**Table IV.** Bactericidal activity of triclosan 7.5 mg/L on exponentially growing, stationary phase and non-growing cultures of *S. aureus* NCTC 6571, MRSA 9543 and triclosan-resistant mutant TM1

<i>S. aureus</i> strain	Log <sub>10</sub> reduction in cfu after exposure to triclosan 7.5 mg/L											
	exponential phase				stationary phase				non-growing			
	30 s	5 min	10 min	20 min	30 s	5 min	10 min	20 min	30 s	5 min	10 min	20 min
NCTC 6571	0.05	0.89	1.74	3.97	0.10	0.93	1.82	4.30	0.14	1.04	1.65	4.12
MRSA 9543	0.12	0.94	1.97	4.17	0.09	1.04	1.90	4.05	0.25	1.03	1.81	4.14
TM1	0.14	0.81	1.68	3.81	0.00	0.85	1.69	3.79	0.05	0.88	1.91	4.09

## Discussion

Triclosan is being used in an increasing range of products and its use as a whole-body wash has played a valuable role in the control of MRSA outbreaks,<sup>13</sup> either alone or in conjunction with the application of mupirocin to the anterior

nares of nasal carriers of MRSA.<sup>1,14</sup> Strains of *S. aureus* with low-level triclosan resistance (1–4 mg/L) have been isolated.<sup>7,15</sup> However, Bamber & Neal<sup>8</sup> have recently isolated hospital strains with MICs of up to 32 mg/L. They suggested that the final concentration of triclosan in triclosan baths will be approximately 4–5 mg/L, lower than



**Figure.** Triclosan-induced potassium leakage from (a) *S. aureus* NCTC 6571, (b) methicillin-resistant *S. aureus* 9543 and (c) TM1 (triclosan-resistant mutant derived from NCTC 6571). Cultures were exposed to triclosan 15 (▲), 7.5 (■) or 2 mg/L (◆).

the MIC of some of the strains tested; this could result in a failure of triclosan baths to eradicate MRSA from the colonized patient. In our study, however, with 32 clinical isolates of *S. aureus* resistant to one or more antibiotics, the highest MIC observed was 1 mg/L, and the lowest 0.025 mg/L for the most sensitive strains. The clinical significance of the low-level triclosan resistance seen in the majority of the strains tested is unknown.

Mupirocin-resistant *S. aureus* strains have also emerged, the determinant for which is carried on a transferable plasmid. Cookson *et al.*<sup>7</sup> isolated MRSA strains that exhibited low-level resistance to triclosan (2–4 mg/L) from patients using mupirocin in addition to triclosan baths. This triclosan resistance was shown to be transferable in association with the plasmid-mediated mupirocin resistance, although co-resistance to the disinfectants chlorhexidine, quaternary ammonium compounds, povidone iodine and acridine was not observed. The triclosan resistance could also be cured by incubation at 42°C, indicating that it was associated with

the plasmid. It is possible that the intensive use of triclosan will result in an increased frequency of triclosan-resistant *S. aureus*. In view of this, it may be advisable routinely to test isolates for their triclosan sensitivity as is the case for mupirocin.

It is thought that strains with decreased susceptibility to disinfectants may also be less susceptible to antibiotics,<sup>16</sup> possibly because of common resistance mechanisms. The use of disinfectants might, in theory, select for strains resistant to antibiotics, and vice versa. Some of the strains that were resistant to numerous antibiotics also showed low-level resistance to triclosan, although there were exceptions. In order to be able to draw any positive conclusions as to the potential of cross-resistance between triclosan and antibiotics, large-scale testing of isolates from various sources is required.

Bamber & Neal<sup>8</sup> demonstrated that low-level triclosan resistance amongst *S. aureus* clinical isolates was observed in equal frequencies whether the organism was sensitive or resistant to methicillin. In addition, none of their isolates that exhibited mupirocin resistance had decreased susceptibility to triclosan. Similarly, we were unable to increase the resistance of *S. aureus* to triclosan by the transfer of a plasmid encoding mupirocin resistance to triclosan-sensitive strains, in contrast to the work of Cookson *et al.*<sup>7</sup>

Three issues associated with triclosan use need to be considered in evaluating triclosan resistance: (i) the presence of triclosan at low concentrations in the environment; (ii) an understanding of the mechanism(s) of triclosan resistance and how this influences the possible development of resistance to other biocides and to antibiotics; and (iii) an understanding of its mechanism of antibacterial action.

Triclosan is known to be stable in the environment and may be present on surfaces at low concentrations when used as a disinfectant. There is concern that continuous exposure to low (sub-MIC) concentrations of triclosan may give rise to organisms with enhanced resistance to the disinfectant and/or antibiotics. This concept remains to be demonstrated. The inability to develop enhanced resistance to triclosan in the present investigation is in agreement with other studies undertaken.<sup>17</sup>

Those strains that were selected for decreased sensitivity to triclosan did not exhibit any increase in resistance to the antibiotics vancomycin, methicillin, penicillin, gentamicin, erythromycin or tetracycline. However, this elevated triclosan resistance was stable in six out of 10 mutants tested in the presence or absence of triclosan (acting as a selective pressure). This may have important clinical implications where the concentration of residual triclosan may vary on different surfaces over different periods of time. Attempts to isolate further mutants with even higher triclosan MICs failed. The clinical implications of low-level resistance to triclosan are unknown.

Until recently triclosan has been regarded as a biocide with a range of cytoplasmic membrane and intracellular

target sites. One such target is the enzyme enoyl reductase, encoded by the *FabI* gene in *Escherichia coli*.<sup>10,18</sup> Enoyl reductase uses NADH to reduce double bonds during fatty acid elongation and thus is a major component of lipid synthesis. Backed by mutational and some biochemical analyses in *E. coli* and *Mycobacterium smegmatis*,<sup>19</sup> claims have been made that this is the sole target of triclosan in those organisms.<sup>2,10,20</sup> Heath *et al.*<sup>18</sup> suggest that the observed perturbation to the cytoplasmic membrane arises indirectly as a consequence of the action of triclosan on this enzyme. If the sole target of triclosan is this enzyme involved in lipid synthesis, then should exponentially growing organisms be more susceptible to it? This was not observed in this study where *S. aureus* was equally sensitive to triclosan whether in the exponential or stationary phase of growth. Furthermore, non-growing cells were inactivated to the same degree as cells in a growth medium. Alternatively, McDonnell & Pretzer<sup>11</sup> argue that, although enoyl reductase may be a major target, triclosan also has multiple target sites, primarily within the cytoplasmic membrane, that inhibit lipid, RNA and protein synthesis, and result in direct membrane damage and cell death. Triclosan-induced cytoplasmic membrane damage resulting in the loss of intracellular potassium ions from washed suspensions of cells occurred at a similar rate in *S. aureus* NCTC 6571, TM1 and MRSA 9543 despite the differences in their triclosan MICs (0.025, 1 and 1 mg/L, respectively). The lack of correlation between MICs and lethal effects has been demonstrated previously,<sup>7,21,22</sup> and is presumably a result of the mechanism of action of triclosan. Generally, antibiotics have single target sites and consequently increased MICs and reduced bactericidal effectiveness are linked. In contrast, biocides have multiple targets, and increased MICs often do not correlate with decreased bactericidal activities of that compound.<sup>11</sup> It appears that additional triclosan-induced cellular changes are required to produce a bactericidal effect whether the staphylococcal strain is resistant or sensitive to triclosan as judged purely by the MICs.<sup>23</sup>

The presence of proton-dependent efflux pumps has been proposed as a possible mechanism of decreased susceptibility to biocides in *E. coli*<sup>24</sup> and *Pseudomonas aeruginosa*.<sup>25</sup> AcrAB, an efflux protein found in *E. coli*, is thought to prevent intracellular accumulation of bile but also protects against other compounds such as antibiotics. McMurry *et al.*<sup>24</sup> suggested that mutations at the *acrAB* locus may act synergistically with mutations at other loci (e.g. *FabI*), leading to decreased susceptibility to triclosan. Another proposed mechanism of resistance is cell wall permeability changes that prevent triclosan reaching its target site. *P. aeruginosa* has a complex cell envelope and exhibits intrinsic resistance to triclosan. *S. aureus*, on the other hand, has a less complex cell wall and is more sensitive.<sup>26</sup>

In many instances, triclosan is incorporated into products (e.g. bath products) that have other ingredients, such

as surfactants and chelators, that promote cell damage. These ingredients may also affect resistance by placing additional stress on the bacteria and so they must be critically evaluated in *in-vitro* tests when preparations other than pure compounds are being evaluated. The efficacy of antimicrobial products may depend on, and vary significantly with, the formulation used.<sup>27</sup>

The use of triclosan as a biocide will remain controversial until the mechanisms of resistance and the relative importance of low-level resistance in the environment are better understood. Surveys of the occurrence of resistance to disinfectants in natural settings are needed to determine whether there is a cause for public health concern.

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